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Heteroplacidium compactum reported as a genus new to China

Xiang-Min Cheng[1,2], Da-Le Liu[1,3], Xin-Li Wei*[1,2], Jiang-Chun Wei[1,2]

[1] *State Key Laboratory of Mycology, Institute of Microbiology,*
Chinese Academy of Sciences, Beijing 100101, China
[2] *University of Chinese Academy of Sciences, Beijing 100049, China*
[3] *College of Life Sciences, Southwest Forestry University, Kunming 650224, China*

* Correspondence to: *weixl@im.ac.cn*

Abstract—Three specimens of *Heteroplacidium compactum* (*Verrucariaceae*), isolated from arid areas in Northwest China, were identified by morphological and phylogenetic comparisons with other species, including species of *Endocarpon* and *Placidium* (common verrucariaceous genera in the same area). This is the first report of *Heteroplacidium* from China.

Key words—desert, ITS, lichen, morphology, taxonomy

Introduction

Breuss (1996) separated four genera—*Anthracocarpon*, *Clavascidium*, *Heteroplacidium*, and *Involucropyrenium*—from *Catapyrenium* Flot. s. lat. (*Verrucariaceae*) based on morphological characters. *Neocatapyrenium* H. Harada, *Placidium* A. Massal., and *Scleropyrenium* H. Harada are also morphologically related (Prieto & al. 2010b) but are independent monophyletic lineages within *Verrucariaceae*, according to phylogenetic analyses (Gueidan & al. 2007, 2009; Prieto & al. 2010b).

Endocarpon Hedw. (*Verrucariaceae*) was relatively common during our field survey in the arid region of Northwest China (Yang & Wei 2008, Zhang & al. 2017a), but no species of *Heteroplacidium* Breuss was recorded. *Heteroplacidium*, which grows primarily on soil and rock in warm temperate

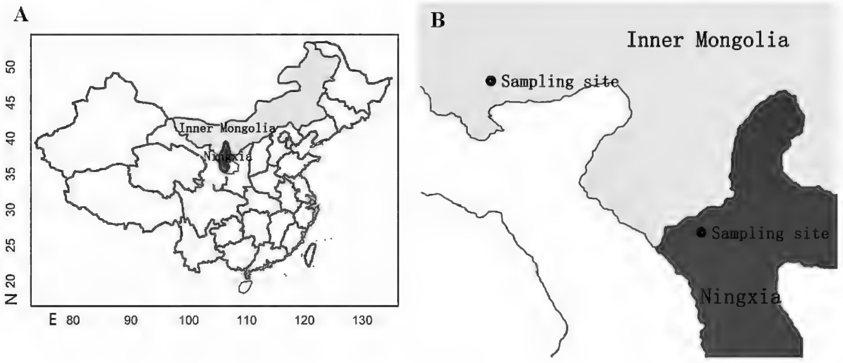

Fig. 1. *Heteroplacidium compactum* collection sites in China.

regions, is characterized by a crustose-areolate to squamulose thallus, a paraplectenchymatous or subparaplectenchymatous cortex, and clavate asci with biseriate ascospores (Prieto & al. 2010a); twelve species have been accepted (Knudsen & al. 2014). We report *Heteroplacidium* in China for the first time, based on three specimens of *H. compactum*.

Material & methods

Phenotypic analysis

All *Heteroplacidium* samples were collected from Ningxia Hui Autonomous Region and Inner Mongolia (Fig. 1) and are preserved in the Lichen Section of Herbarium Mycologicum Academiae Sinicae, Beijing, China (HMAS-L). Zeiss Stemi SV11 dissecting and Zeiss Axioskop 2 plus compound microscopes were used for morphological examinations.

DNA extraction, amplification, sequencing

DNA was extracted from our three fresh specimens of *Heteroplacidium* (Table 1) following the modified CTAB method (Rogers & Bendich 1988). The internal transcribed spacer of nuclear ribosome DNA (nrDNA ITS), the standard DNA barcoding marker (Schoch & al. 2012), was chosen as the genetic marker. Primers LR1 (Vilgalys & Hester 1990) and ITS1 (White & al. 1990) were used. Reactions were carried out in 50 µL reaction volume comprising 3 µL total DNA, 1 µL each primer (10 µM), 25 µL 2×Taq MasterMix (CWBIO) and 20 µL ddH$_2$O. The DNA was amplified in a Biometra T-Gradient thermal cycler as follows: initial heating for 5 min at 95 °C; 35 cycles of 30 s at 94 °C, 30 s at 56 °C, and 90 s at 72 °C; ending with a final extension of 8 min at 72 °C, after which the samples were kept at 4 °C. Negative controls were prepared for each amplification series. PCR products were purified using a CWBIO gel extraction kit following the manufacturer's instructions.

Sequence alignment and phylogenetic tree construction

PCR products from the three putative *Heteroplacidium* specimens were sequenced using ABI 3730 XL Sequencer. Additional sequences of *Endocarpon* (3 spp.), *Heteroplacidium* (4 spp.), and *Placidium* (3 spp.) were downloaded from GenBank (Table 1) with the *Endocarpon* sequences selected as outgroup. The sequences were aligned using ClustalW Multiple Alignment (Thompson & al. 1994) in BioEdit 7.2.5 (Hall 1999). The program Gblocks v0.91b (Castresana 2000, Talavera & Castresana 2007) was used to delimit and remove regions of alignment uncertainty, using options for a "less stringent" selection on the Gblocks web server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html). We confirmed the phylogenetic position of *Heteroplacidium* sp. represented by our three specimens by subjecting the alignment to a maximum likelihood (RAxML) analysis; nodal support was assessed using 1000 bootstrapping pseudoreplicates with RAxML-HPC v. 8.2.6 (Stamatakis 2014) and MrBayes v.3.2.6 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) on the CIPRES Science Gateway (http://www.phylo.org). In the ML and Bayesian analyses, substitution models for ITS were estimated using jModelTest-2.1.9 (Guindon & Gascuel 2003, Darriba & al. 2012). Based

Table 1. Specimens and sequences of *Endocarpon*, *Heteroplacidium*, and *Placidium* used in the phylogenetic analysis

Species	Voucher	Origin	GenBank No.
E. adscendens	CG671	Switzerland	KF959777
E. pusillum	CG470	—	JQ927447
E. tenuissimum	Lendemer 29447	USA	KM371592
	Lendemer 27013	USA	KM371593
H. acervatum	LI 271015	Spain	GU228954
	M. Prieto 399	Spain	GU228955
H. compactum	M. Prieto 1701	Spain	GU228952
	M. Prieto 1607	Spain	GU228949
	XL2017117	China	MH930459*
	ALS2018002	China	MH930460*
	ALS2018036	China	MH930461*
H. congestum	LI 552268	USA	GU228950
	LI 297536	USA	GU228951
H. divisum	LI 218428	Italy	GU228953
P. pilosellum	M. Prieto 3	Spain	GU228993
	VEL_PLG	Slovakia	KY981585
P. podolepis	LI 297365	Argentina	GU228956
P. squamulosum	M. Prieto 336	Spain	GU228994

* = sequences newly generated for this study by the authors

on these results, we used the TrN+I+G model with 1000 pseudoreplicates in the ML analysis, and two parallel Markov chain Monte Carlo (MCMC) runs were performed in MrBayes, each using 8 million generations and sampling every 1000 steps. A 50% majority-rule consensus tree was generated from the combined sampled trees of both runs after discarding the first 25% as burn-in. Tree files were visualized with FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

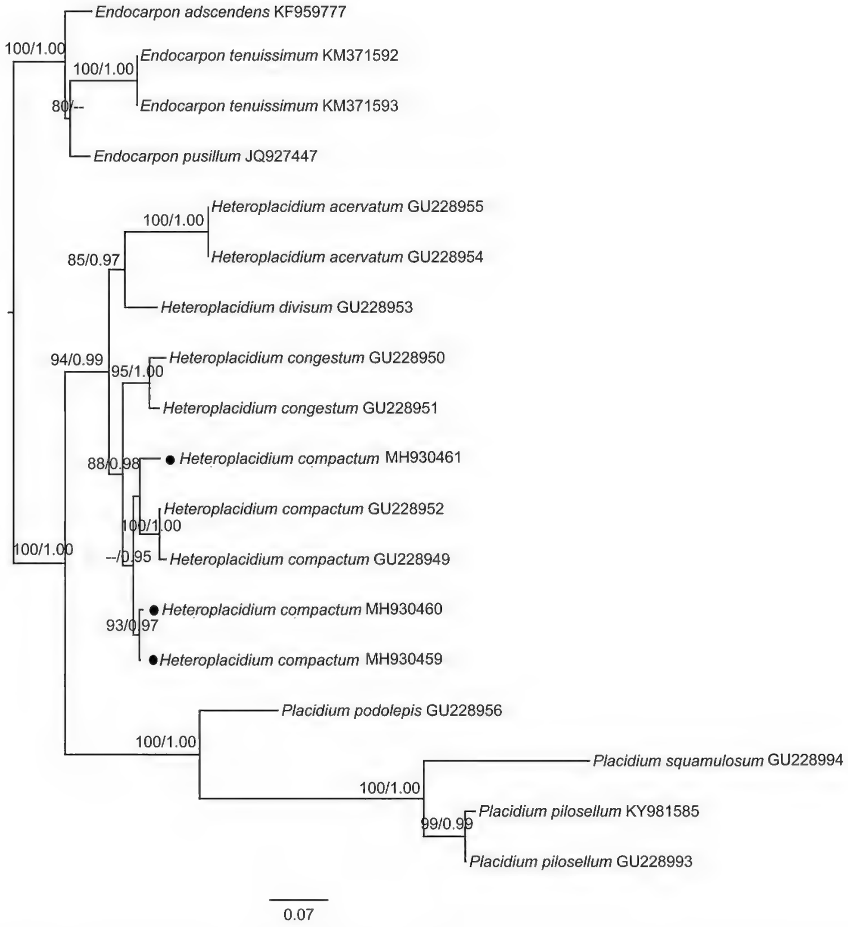
Endocarpon adscendens KF959777
100/1.00
100/1.00
Endocarpon tenuissimum KM371592
80/--
Endocarpon tenuissimum KM371593
Endocarpon pusillum JQ927447
100/1.00
Heteroplacidium acervatum GU228955
85/0.97
Heteroplacidium acervatum GU228954
Heteroplacidium divisum GU228953
94/0.99
95/1.00
Heteroplacidium congestum GU228950
Heteroplacidium congestum GU228951
88/0.98
● Heteroplacidium compactum MH930461
Heteroplacidium compactum GU228952
100/1.00
100/1.00
--/0.95
Heteroplacidium compactum GU228949
● Heteroplacidium compactum MH930460
93/0.97
● Heteroplacidium compactum MH930459
Placidium podolepis GU228956
100/1.00
Placidium squamulosum GU228994
100/1.00
Placidium pilosellum KY981585
99/0.99
Placidium pilosellum GU228993
0.07

Fig. 2. The maximum likelihood tree of *Endocarpon*, *Heteroplacidium*, and *Placidium* species based on ITS sequences. The numbers in each node represent bootstrap support (BS) and posterior probability (PP) values. Bootstrap values ≥75 and posterior probability values ≥0.95 were plotted on the branches of the RAxML tree. Except for the three samples of *Heteroplacidium compactum* marked by the solid circle '●', all other sequences were downloaded from GenBank. Scale bar = 0.07 substitution per site.

Phylogenetic and species delimitation analyses

The aligned matrix contained 455 unambiguous ITS nucleotide positions. The phylogenetic tree (Fig. 2) included 10 taxa representing three genera within *Verrucariaceae*. *Heteroplacidium* formed a well-supported monophyletic clade (BS = 94, PP = 0.99) with the distinct species clearly separated. Our three Chinese specimens all grouped with Spanish sequences of *H. compactum*.

Taxonomy

Heteroplacidium compactum (A. Massal.) Gueidan & Cl. Roux,
Bull. Inf. Ass. Franç. Lichén. 33(1): 25, 2008. Fig. 3

Thallus areolate, areoles 0.6–1.8 mm diam., angular to rounded, flat to slightly convex; upper surface dark brown to black, dull to somewhat shiny.

Thallus layer ≤300 µm thick; upper cortex paraplectenchymous, 12.5–37.5 µm thick; algal layer 62.5–150 µm thick, cells 12.5–17.5 µm diam; medulla 112.5–187.5 µm thick; lower cortex paraplectenchymous, 10–12.5 µm thick or not clearly delimited. Rhizohyphae simple, intersecting,

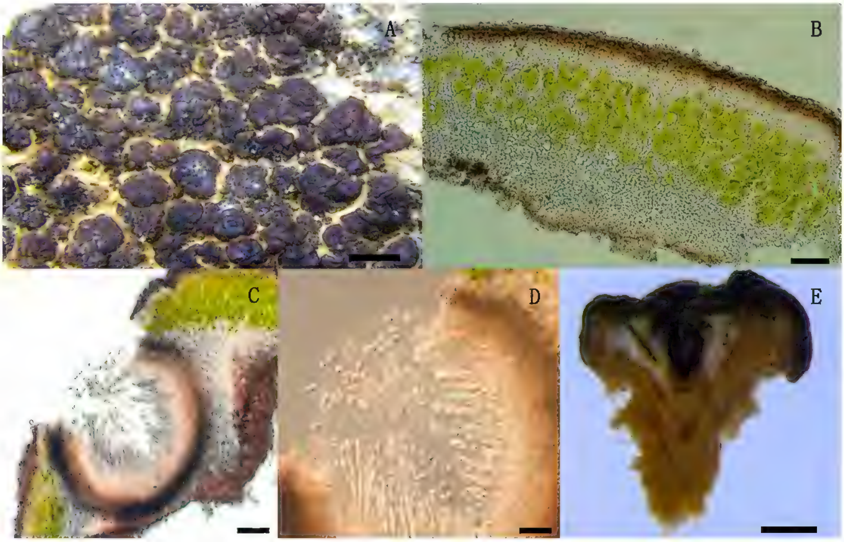

Fig. 3. *Heteroplacidium compactum* (HMAS-L 140900): A. Habit of thallus; B. Anatomical structure of thallus; C. Perithecia in thallus; D. Ascospores; E. Separate areole with abundant rhizines tangling with soil particles. Scale bars: A = 1 mm; B = 20 µm; C = 100 µm; D = 50 µm; E = 0.5 mm.

4.5–7.5 μm diam. PERITHECIA nearly spherical, 300–500 μm diam, exciple, pale darkening with age. ASCI clavate, 52.5–62.5 × 15–17.5 μm. ASCOSPORES simple, ellipsoid, 12.5–17.5 × 10–12.5 μm. PYCNIDIA not seen.

SPECIMENS EXAMINED. **CHINA. INNER MONGOLIA**: 39°28′22″N 101°04′04″E, 1563 m alt., on sand, 5 June 2018, D.L. Liu & al. ALS2018002 (HMAS-L 0141672; GenBank MH930460); 39°32′31″N 101°06′34″E, 1478 m alt., on sand, 5 June 2018, D.L. Liu et al. ALS2018036 (HMAS-L 0141671; GenBank MH930461). **NINGXIA: Zhongwei City**, Cui Liu Gou. 37°24′35″N 104°35′09″E, 1577 m alt., on sand, 15 July 2017, D.L. Liu & R.D. Liu XL2017117 (HMAS-L 140900; GenBank MH930459).

DISTRIBUTION: *Heteroplacidium compactum* has previously been reported from Asia (Mongolian People's Republic; Pakistan), Europe, and northern Africa (Breuss 1994; Prieto & al. 2010a). In China, it is now known from arid desert areas in Inner Mongolia and Ningxia.

Discussion

The characters of Chinese *H. compactum* are essentially similar to the previous descriptions (Breuss 1994; Prieto & al. 2010a) in the areolate thallus, dark brown upper surface, paraplectenchymous upper and lower cortex, and the shape and size of the perithecia and asci. Among the differences noted are a thinner thallus (300 μm versus 600 μm) and broader ascospores (10–12.5 μm versus 8-10 μm). Furthermore, pycnidia were not seen in the Chinese materials.

In the arid desert area of China, *Endocarpon* is quite common and comprises a key component of biological soil crust communities (BSC). The biodiversity reported for these Chinese BSCs includes several newly described *Endocarpon* species (Yang & Wei 2008, Zhang & al. 2017a) with the predominant species, *E. pusillum*, studied physiologically (Zhang & Wei 2011), ecologically (Ding & al. 2013), and genomically (Wang & al. 2014, 2015, Li & Wei 2016, Zhang & al. 2017b). *Heteroplacidium* (with *Dermatocarpon*-type pycnidia) is clearly different from *Endocarpon* (with *Endocarpon*-type pycnidia).

The most similar genus to *Heteroplacidium* is *Placidium* A. Massal., which is also characterized by *Dermatocarpon*-type pycnidia, but the two genera have differently sized thallus squamules (*Heteroplacidium* 0.3–3 mm diam; *Placidium* 2–8(–15) mm diam). In the phylogenetic tree (FIG. 2), *Heteroplacidium* is most closely related to *Placidium*, clustering in a well-supported clade (BS = 100, PP = 1) and relatively distant from *Endocarpon*.

Acknowledgments

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